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## THE TWINNING AND MONEMBRYONIC DEVELOPMENT OF PLATYGASTER HIEMALIS, A PARASITE OF THE HESSIAN FLY [1]


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### INTRODUCTION

Those who are familiar with the polyembryonic method of development of some insects have always thought it desirable that a study be made of the development of a species which produces but a small number of individuals from a single egg. In the four species of insects in which the polyembryonic method of development has been previously described in greater or less detail, the number of individuals produced from a single egg has been 150 to about 1,700. These species represent a highly specialized type of polyembryony, whereas a species in which one egg produced only two or four individuals would be expected to illustrate a more simple type of polyembryony.

*Platygaster hiemalis* Forbes, a hymenopterous parasite of the Hessian fly,[3] is a species which develops both monembryonically and polyembryonically. This parasite deposits a group of from 5 to 8 eggs in the egg of its host, and an average of 6.31 individuals are developed from these eggs. Some of the eggs of the group fail to develop at all, others of the group develop twins, while still other eggs of the same group give rise to single individuals. The objects of this paper will be to describe the twinning and monembryonic development of this insect, to show why some of the eggs fail to develop, and to point out the relation between the more simple type of polyembryony here called twinning and monembryony.[4]

### RELATION OF PARASITE TO HOST

*Platygaster hiemalis* is single-brooded, although its host, the Hessian fly, a serious wheat pest, is double-brooded throughout most of the winter-wheat region of the United States. The adults of *P. hiemalis* emerge from their cocoons in the host puparia (Pl. 1, D) during the fall of the year, at which time the Hessian flies of one generation also emerge. The parasite deposits its eggs in the eggs of the fly which have meanwhile been deposited on the wheat plants.


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[2] The authors desire to express their appreciation of the interest shown by Dr. L. O. Howard and W. R. Walton in the studies reported in this paper.

[3] *Phytophaga destructor* Say.

[4] A detailed account dealing with the bionomics of this parasite and its economic importance as an enemy of the Hessian fly is being prepared by the junior author.

The early development of the parasite takes place in the host egg and in the young host larva during fall (Pl. 1, C, E), the winter being passed in the embryonic stage within a well developed host larva, which remains on the wheat plant. In spring the Hessian fly larvæ, if unparasitized, soon produce adult flies. Meanwhile, the parasites in parasitized host larvæ complete the embryonic stage of development, and remain in this condition in the fully grown host larvæ until early summer. During July the newly developed parasite larvæ mature by feeding upon the body content of the host. The parasite larvæ then pupate in individual cocoons made within the host puparium, the adult parasites finally emerging from their cocoons in the fall.

## DEVELOPMENT OF THE EGG TO CLEAVAGE

The precleavage development of the egg of but one species of the Platygastridae (*Platygaster dryomyiae* Silv.) which develops by the monembryonic method has been described by Silvestri (*11*).[3] This phase of the development of polyembryonic Hymenoptera has been demonstrated by Silvestri (*9*) and Patterson (*5*) in *Copidosoma truncatellum* and by Leiby (*2*) in *Copidosoma gelechiae*. In all cases it has been shown (1) that two maturations of the oocyte nucleus occur, (2) that the two resulting polar bodies are not thrown off (as they usually are in monembryonically developing eggs) but are retained in the anterior or polar region of the egg, (3) that the polar region is differentiated from the posterior or embryonic region, and forms together with the polar bodies a nucleated membrane which encompasses each embryo in the course of its development, and (4) that the fertilized or unfertilized cleavage nucleus divides and is destined eventually to give rise to the embryos. The processes by which the precleavage stages of these eggs take place differ only slightly, but the later stages of development of the demonstrated species are known to differ very markedly.

### THE NEWLY DEPOSITED EGG

The eggs of *Platygaster hiemalis* are regularly deposited in groups of from 4 to 8, at each oviposition in the host egg. Hence they are frequently found in contact and side by side (Pl. 1, A), or very close to each other. It, therefore, is not difficult to ascertain whether a host egg has been oviposited in more than one time, or how many eggs are deposited at a single oviposition.

The newly deposited egg (Pl. 1, E) is ovoid in shape, usually bluntly rounded at the posterior end, and somewhat pointed at the anterior end. Sectioned eggs measure 15$\mu$ in length and 6$\mu$ in width, or 5$\mu$ in length and 2$\mu$ in width less than freshly deposited unfixed eggs. The cytoplasm of the egg is densely and finely granular, homogeneous throughout, and without oil spherules or vacuoles. The presence of a weakly defined membrane or chorion becomes evident in eggs that shrink considerably following some fixations.

The egg nucleus is spherical and measures 2.5$\mu$ in diameter. It is regularly found in or very near the center of the egg. When sectioned the nucleus of the newly deposited egg exhibits its chromosomes scattered over a reticulum.

[3]Reference is made by number (italic) to "Literature cited," p. 349.

If the egg is inseminated the sperm can be seen usually in a curved or arched position (Pl. 1, E; Pl. 2, A–C), and frequently extending more than two-thirds the length of the egg. The body part and tail of the sperm measure 2.7$\mu$ and 7.3$\mu$, respectively. Never more than one sperm has been found in one egg. It is quite evident that not all of the eggs deposited at one time by a fertilized parasite contain sperms. This point has been studied thoroughly, careful examinations of groups of eggs showing that about one-third of the eggs are not inseminated. For example, only 5 of the group of 8 eggs shown at F on Plate 1 contain sperms.

There is no evidence whatever of the presence of a nucleolus or so-called germ-cell determinant. Nor did Silvestri find this nuclear body in *Platygaster dryomyiae*, although it has been recorded as present in the eggs of polyembryonic Hymenoptera previously described.

## MATURATION

Both fertilized and unfertilized eggs maturate and develop adult parasites, the fertilized eggs presumably giving rise to females and the unfertilized eggs developing males; although on this point no conclusive biological or cytological evidence is available. The process of maturation is identical in fertilized and unfertilized eggs.

During the first hour after oviposition the oocyte nucleus (Pl. 1, E; Pl. 2, A) increases slightly in size and becomes clearer, whereupon the chromosomes are seen scattered over a reticulum. The nucleus then becomes more concentrated (Pl. 2, B) and is found immersed in a semiclear area of the oocyte cytoplasm. During this time its chromatin appears as a condensed mass of fine granules. Two hours after oviposition the nucleus again expands slightly (Pl. 2, C) and chromatin bodies are to be seen supported at the nodes of the reticulum. At four hours the nucleus is found at prophase, in the act of forming a spindle, or at anaphase (Pl. 2, D–F). The spindle is always arranged longitudinally in the egg. At the close of the first maturation the first polar body is always found at the anterior pole while the oocyte nucleus of the second order is found near the center of the egg (Pl. 2, G).

Previous to the second maturation, which takes place at about the seventh hour, the first polar body becomes concentrated and appears as an irregular homogeneous mass of chromatin that measures about 1$\mu$ in diameter. Between the fourth and the seventh hours, the oocyte nucleus of the second order undergoes changes similar to those described for the nucleus previous to the first maturation; and at the end of the seventh hour the nucleus is found at prophase (Pl. 2, J). The spindle which is then formed is again disposed longitudinally in the anterior half of the egg (Pl. 2, L, M).

After the second division, the second polar body is seen in the anterior end, near or adjacent to the first polar body; while the oocyte nucleus of the third order (later the female pronucleus) remains in the center of the egg until about the ninth hour, after which it may be found almost anywhere in the posterior half of the egg. From the eighth to the sixteenth hour the female pronucleus is apparently in a resting stage, although during this interval it increases in diameter from 1.8 $\mu$ to 3.5 $\mu$.

## FERTILIZATION

Prior to first maturation of the oocyte nucleus, the sperm is found in any part of the inseminated egg; but shortly afterward it assumes the appearance of a somewhat elongate or crescentic nuclear body (Pl. 2, A–I). It is regularly found thereafter in the posterior half of the egg.

After the second maturation the male nucleus becomes spherical, stains deeply, and measures 1.5 μ in diameter (Pl. 2, K–P). At the eleventh hour the male pronucleus expands until it measures 2 μ in diameter. At about the twelfth hour the male and female pronuclei are found regularly close to each other (Pl. 2, R–T) in the posterior part of the egg. Fusion of these two nuclei (Pl. 2, U) is effected at about the sixteenth hour. The egg now contains a polar nucleus in the anterior region and a single cleavage nucleus located in the posterior region (Pl. 2, W).

## ORIGIN OF THE PARANUCLEUS

During the development of the embryos rounded or crescentic nuclear masses (Pl. 3, A–F) are found in the nutritive membrane (trophamnion) which surrounds the germs and blastulas. These were termed paranuclear masses by Marchal (*3*) in a species of Encyrtus that develops by polyembryony. Silvestri (*9*, *10*, *11*), Patterson (*8*), and Leiby (*2*) have shown in other polyembryonic Hymenoptera that the paranuclear masses have their origin in the polar bodies. Paranuclear masses having a similar nutritive function are not confined to the polyembryonic Hymenoptera; for they have also been demonstrated by Marchal (*4*) in the monembryonically developing species of Synopeas, Trichasis, and *Platygaster ornatus*.

The two polar bodies developed in the course of maturation in *Platygaster hiemalis* also give rise to a polar nucleus or paranuclear mass. At the close of the eighth hour they lie close to each other in the anterior end of the egg (Pl. 2, N), where they are recognized as compact, darkly staining masses of chromatin measuring approximately 0.7 μ and 1.0 μ in diameter, respectively.

The first polar body, unlike those in all other polyembryonic Hymenoptera previously described, does not divide during second maturation. Instead, the two polar bodies fuse to form a single polar nucleus (Pl. 2, O–Q) at about the tenth hour. This behavior of the polar bodies differs from that shown by Silvestri (*11*), in *Platygaster dryomyiae*, in which he found that the first polar body divides, and that the anterior half of the divided first polar body forms one polar nucleus, while the posterior half of the first polar body and the second polar body fuse to form a second polar nucleus.

Between the tenth and twelfth hours the polar nucleus elaborates until it fills most of the anterior region of the egg and measures about 4.5 μ in diameter (Pl. 2, R). It remains a single more or less spherical nucleus (Pl. 2, S–V) until just before the division of the cleavage nucleus, when it divides amitotically to form two subequal paranuclear masses (Pl. 3, A).

## DIFFERENTIATION OF EMBRYONIC REGION AND FORMATION OF TROPHAMNION

About the twenty-fourth hour two regions are recognized in the egg, when the central part of its posterior half becomes distinctly differentiated

from the remainder of the egg (Pl. 2, X). This differentiated region contains the cleavage nucleus, and is called the embryonic region, since it later gives rise to the embryos. The remainder of the egg containing the polar nucleus constitutes the polar region.

Between the first and second days the group of from 5 to 8 eggs deposited by the parasite in the host becomes somewhat dispersed throughout the developing host if they were deposited in a host egg. If the eggs were deposited in an embryo well advanced in development at the time of oviposition or in a recently hatched host larva, they become scattered in the body cavity of the host at an earlier hour. In any event the eggs begin to increase in size soon after the first day, when they measure 18.2 $\mu$ in length and 7.8 $\mu$ in width. Since the eggs increase in size, the egg stage may be regarded as past and henceforth the eggs may be denoted as parasite bodies.

During the dispersal of the parasite bodies in the cavity of the host they become lodged against the host tissues, such as the salivary glands or fat tissues, whereupon portions of these tissues soon encompass the parasite bodies. Occasionally two parasite bodies may be surrounded by the same bit of host tissue, but both parasite bodies will usually continue to develop independently of each other. After each parasite body is surrounded (at least partially) by host tissue, an elaboration of the paranuclear masses in the polar region can immediately be observed. This elaboration is obviously due to the absorption of host tissues by the parasite body through its polar region. The polar region, including that part which surrounds the embryonic region, is therefore properly known as the trophamnion.

## CLEAVAGE TO BLASTULA STAGES

The development of the embryos, from cleavage of the segmentation nucleus to formation of the blastulas, takes place for the most part in the young host larva, or during the interval from the second to the tenth day after oviposition. In some sectioned host larvæ the blastula stage of the parasites is found at the end of the sixth day, but this apparent precocious development occurs only in host larvæ which were about ready to hatch from the host egg at the time of oviposition by the parasite.

### FIRST AND SECOND CLEAVAGE

The first cleavage of the segmentation or embryonic nucleus takes place at about the second day (Pl. 3, B). On the third day cleavage is found completed and two embryonic nuclei are visible in the embryonic region (Pl. 3, C). Each embryonic nucleus measures 3.8 $\mu$ in diameter, or about the same size as the original segmentation nucleus. The two paranuclear masses are larger; one of the masses is seen migrating from the anterior end to that part of the trophamnion which surrounds the embryonic region. The entire parasite body has increased slightly in size during the second day, and now measures about 19.2 $\mu$ in length and 9.1 $\mu$ in width.

The second cleavage of the embryonic nuclei takes place between the fourth and fifth days and produces four nuclei in the embryonic region (Pl. 3, D). Meanwhile the embryonic region has become spherical, and has increased in diameter to 10 $\mu$. One of the paranuclear masses is now found beside the embryonic region.

During the fifth day the structure of the parasite body remains the same, but there is a notable increase in size of the parasite body, so that by the end of the fifth day the parasite body measures 22 μ in length and 11.4 μ in width (Pl. 3, F). The paranuclear masses are larger and usually widely separated in the trophamnion. At this time the parasite body is entirely surrounded by host tissue. This condition is true at least of most of the parasite bodies in a particular host (that is, say, four of six eggs originally deposited in the host), but others are to be seen in the same host which are not surrounded by host tissue, these being in a retarded and inactive stage of development. The retarded developing forms will be referred to later.

## DIVISION WITHIN THE PARASITE BODY TO FORM TWIN GERMS

About the sixth day after oviposition the parasite body departs from its previous oval shape and becomes more elongate. The assumption of this newer shape is due to a division of each of the two paranuclear masses to form four separate masses. Two paranuclear masses are now to be found near or at each end of the parasite body.

A division of the embryonic region in some of the parasite bodies to form two separate embryonic regions then takes place, two of the four embryonic nuclei becoming included with each half of the divided embryonic region (Pl. 3, G). Each half of the embryonic region is now a true germ and is structurally the same as a 3-day-old parasite body after the first cleavage, since it is composed of two paranuclear masses and two embryonic nuclei in an embryonic region. No further division of the embryonic region or later developing blastula stages takes place; but each germ develops directly into the blastula and late embryo stages, and finally into a larva. The original egg (or parasite body) therefore develops into twin parasites.

## SEPARATION AND DEVELOPMENT OF GERMS

Immediately after the division of the embryonic region to form twin germs, the trophamnion of the parasite body is still continuous (Pl. 3, H). Indentations of the trophamnion between the twin germs are soon visible, however (Pl. 3, G), and the two germs finally become structurally independent of each other. Henceforth each germ continues development by itself, although for a while both germs may continue to develop side by side until the blastula, or even a later stage, is reached, the two germs being held in contact or close together solely by the host tissue which surrounds them.

After the twin germinal regions are formed, the two embryonic nuclei in each germ (Pl. 3, G) divide and give rise to four embryonic nuclei (Pl. 3, H; Pl. 4, A). The embryonic region of each germ is spherical and measures about 12 μ in diameter when the four embryonic nuclei are just formed. The next cleavage of the embryonic nuclei results in the production of 8 nuclei in each germ. After the third cleavage, when 16 embryonic nuclei are to be found (Pl. 4, B), the nuclei become arranged in a circle (in section), thus assuming the typical early blastula stage. At this stage the embryonic region is still spherical and measures about 19.1 μ in diameter.

During the three cleavages of the embryonic nuclei the embryonic region of the germs is surrounded by the trophamnion. In the tro-

phamnion are two paranuclear masses that usually appear decidedly crescentic in shape (Pl. 4, A, B). The paranuclear masses continue to increase in size through their absorption of elements from the host tissue which surrounds the entire germ or early blastula.

## THE ABORTION OF SOME EGGS

It has been shown above that on an average six to seven eggs are deposited by the female parasite in a host egg or newly hatched larva. If each of these developed twins successfully, the number of parasites produced would always be equal to twice the number of eggs deposited. Hence the average number of parasites reared from host puparia should be 12 or 14. It has been shown by the junior author (*1*), however, that, on the basis of the rearings of 100 puparia, the average number of cocoons produced in a single puparium is 6.52 and the average number of parasites reared is 6.31 per puparium.

One of the reasons why the number of parasites actually reared does not more nearly approximate 12 or 14 is the failure of some of the eggs to develop beyond the maturation and fertilization stages. It is common to find eggs in a stationary or degenerate stage of development at a time when other eggs in the same host, deposited at the same time,[6] are about to twin or have already passed the twinning stage. Figures E, F, and G of Plate 3 represent three types of eggs or parasite bodies found in a single host that originally contained five parasite eggs. Two of these five eggs have reached the twinning stage (one shown at G on Pl. 3); one egg has reached the stage in which it contains two paranuclear masses, and its embryonic region has not as yet divided (Pl. 3, F); and the fourth and fifth eggs have not developed to the stage of first cleavage of the segmentation nucleus (one shown at E on Pl. 3). Eggs of the latter type become aborted. They fail to develop, apparently because they did not become enveloped by host tissues from which they could receive the nutriment necessary to continue their development. At any rate, parasite bodies that are in the normal course of development at the cleavage or later stages are always observed surrounded by host tissue.

Aborted or degenerating eggs nearly always stain less deeply than normal eggs; their nuclei are smaller and frequently appear irregular around the edges, while the nuclei of normal eggs are regularly rounded. Approximately one-third of the eggs deposited by the parasite at one time become aborted.

## MONEMBRYONIC DEVELOPMENT OF SOME EGGS

If twin germs were regularly produced from all nonaborted eggs, an even number of germs or blastulas should always be found in each host larva. That twinning is not the only process of development in this species is indicated by the finding of an odd number of germs or blastulas in perfect serial sections of parasitized larvæ. As examples, two sectioned hosts show seven germs each, a third shows seven blastulas, several show three blastulas, and others show either an odd or even number of both the germ and blastula stages of the parasite.

Moreover, a further study of the stage of the parasites in a host containing an odd number of parasites shows that the parasite bodies

[6] Oviposition into the host was observed under the binocular microscope and in most of the instances by far, only one oviposition was permitted in each egg.

within it are not all in the same stage of development. For example, one host larva containing three parasite bodies will show (1) that two of the parasite bodies are in the twin-germ stage, each embryonic region of which contains four embryonic nuclei (Pl. 3, H), and (2) that the third parasite body contains eight embryonic nuclei in an undivided embryonic region (Pl. 3, I). An older host larva containing seven germs or blastulas will show (1) four blastulas at the 16 or 32 cell stage, which by their paired association in the host and the fact that each pair is held together by host tissue are known to have developed by the twinning process, and (2) three isolated parasite bodies showing 8 or 16 nuclei in their embryonic regions, which by their shape (Pl. 4, C) and position in the host obviously developed from individual parasite eggs. Since twinning of a parasite body takes place only at the 4-cell stage of the embryonic region, and since an odd number of germs and blastulas is frequently found in the host, the conclusion can safely be drawn that some of the eggs of *Platygaster hiemalis* develop monembryonically.

The monembryonic process of development in *Platygaster hiemalis* is not unlike that described by Marchal (4) for the platygastrids *Synopeas rhanis*, *Trichasis remulus*, and *Platygaster ornatus*. In these species Marchal demonstrates how the embryo develops in a differentiated region of the egg, just as *P. hiemalis* does when it develops monembryonically. Moreover, the eggs of the above-mentioned species described by Marchal develop in a cyst of host tissue, nourishment from which is obtained by the trophamnion and the paranuclear masses and supplied to the growing embryo exactly as in *P. hiemalis*.

## SUMMARY AND DISCUSSION OF CLEAVAGE TO BLASTULA STAGES

It has been shown above that after maturation and fertilization in the case of an inseminated egg an embryonic region becomes differentiated in the posterior part of the egg, and that after some growth of the parasite body, the embryonic region divides, two of the four embryonic nuclei passing to each of the two newly produced embryonic regions. Each of these two embryonic regions, together with its component paranuclear masses and trophamnion, develops into the blastula and finally into the larva stage, thus demonstrating a form of polyembryony in this species known as twinning. Moreover, certain of the eggs fail to develop beyond the segmentation nucleus stage and become aborted, probably because they do not become invested by host tissues from which they could absorb nourishing material necessary for their continued development. It has further been shown that some of the parasite bodies do not twin, but on the other hand develop monembryonically in a manner not unlike the monembryonic development described by Marchal for other platygastrid species.

SPECIALIZED MONEMBRYONY.—It is obvious that *Platygaster hiemalis* exhibits both the highest type of monembryonic development and the simplest type of polyembryonic development yet known. In its monembryonic development, it is specialized to the extent that it must draw upon its host for nutriment during the course of development of the embryo, for the reason that its egg is too small (its yolk is therefore insufficient) to develop a larva to the point where it can feed for itself. Consequently the cortex of the differentiated embryonic region is utilized as a nutritive membrane (trophamnion), which, together with its paranuclear masses (of polar body origin), provides nutriment sufficient to

permit full development of the embryo at the expense of, but not to the detriment of, the host.

TWINNING AND SPECIALIZED POLYEMBRYONY.—In its twinning form of development, *Platygaster hiemalis* exhibits the simplest type of polyembryony, in that only two individuals are developed from a single egg or parasite body. Other polyembryonic Hymenoptera, such as *Copidosoma truncatellum* and *Copidosoma gelechiae*, develop 1,500 to 2,000 and 150 to 225 individuals, respectively, from a single egg. In these species the embryonic region does not divide at the end of the second cleavage of the segmentation nucleus, but instead the segmentation nucleus continues to divide within the original embryonic region, until it is a veritable syncytium composed of 200 or more embryonic nuclei. Then at a later stage, the trophamnion surrounding the embryonic region penetrates among the embryonic nuclei, and after portions of the trophamnion encompass one or two embryonic nuclei, the typical germs are formed. Disjunction of the many germs then takes place in a manner similar to that in the twin germs of *P. hiemalis*, but on a larger scale.

*Platygaster hiemalis* further differs from *Copidosoma gelechiae* and *C. truncatellum* in that the germ stage and later stages of *hiemalis* do not divide, while the morula stages of both *gelechiae* and *truncatellum* do divide. It has been shown by Leiby (2) that the morulas of *gelechiae* divide once, each daughter component finally developing into a parasite larva. The senior writer has also observed that the morula stage of *truncatellum* divides once and that the daughter morulas also divide, each tertiary morula giving rise to a parasite larva. Hence it is evident that the division of the morula stage in *gelechiae* and *truncatellum* is simply a specialization of the twinning process exhibited by *hiemalis*. This specialization by division results in the production, from a single egg, of a greater number of parasite individuals which the larger host is able to mature.

ABORTED EGGS AND PSEUDO-FORMS.—As previously pointed out, of the six to eight eggs deposited in the host at the same time by *Platygaster hiemalis*, approximately one-third fail to develop beyond the segmentation nucleus stage, apparently because such eggs fail to become invested by host tissues. Such eggs degenerate. No stages beyond the egg ever degenerate, however, because the twinning and monembryonic processes of development are relatively simple. On the other hand, the complex and more specialized development of the parasite body of *Copidosoma gelechiae* and that of *C. truncatellum* results in the production of some degenerate germs, embryos, and larvæ [7] in these species, along with the normal forms that are destined to mature adult parasites.

It is probable that the development of other polyembryonic species will be described in the future, in which it will be shown that four, eight or more larvæ develop from a single egg. Such species will doubtless show that the number of degenerate or pseudo-forms of the embryonic stage developed will increase in proportion to the number of mature individuals originating from a single egg.

MONEMBRYONY AND THE ORIGIN OF MIXED BROODS.—The origin of single individuals of a sex different from that of all the other individuals reared in the same brood has been of some interest to those who have reared polyembryonic insects. Especially is this true of the species of

[7] These have been described by Leiby (2) in *Copidosoma gelechiae* as pseudogerms, pseudomorulas, pseudembryos, and pseudolarvæ. Similar forms have been observed by the senior writer in *C. truncatellum*.

Platygaster, which emerge in relatively small numbers from an individual host specimen. For example, on an average 6.31 adults of *P. hiemalis* issue from a single host carcass; and the mixed broods frequently show a sex ratio such as five females and one male, or seven females and three males. The rearings of 100 parasitized puparia show that 20 contained no males (pure female broods); and of the 80 mixed broods 23 contained one male, 25 contained two males, 12 contained three males, and 20 contained four or more males. In 71 of the 100 puparia the females exceeded the males in number, in 24 the males exceeded the females, while from the other five host puparia the males and females were reared in equal numbers. Unpublished studies on *Platygaster* sp. are available in notes of the senior writer which show a similar sex ratio.

Patterson (*6, 7*) has also pointed out the sex ratio of *Platygaster felti* Fouts, but in this species the average number of parasites (15 per brood) issuing from a single carcass is nearly three times as great as in *P. hiemalis*. *P. felti* oviposits one or two eggs in a single host egg and not from six to eight, as does *P. hiemalis*. The probabilities are that *felti* develops more than two parasites from a single egg, thus carrying the polyembryonic method of development beyond the twinning stage.

With reference to the development of mixed broods in *Platygaster felti*, Patterson has proposed the theory that both sexes arise from a single egg. He believes that the most probable way that this will be found to occur is by the Bridges method of somatic nondisjunction of the sex chromosome during cleavage of the embryonic nuclei.

The writers believe that the mixed broods of *Platygaster hiemalis* can be readily accounted for by the fact that both fertilized and unfertilized eggs are deposited in the host at the same time,[8] a condition which has been shown to occur regularly. The writers do not believe that this parasite controls insemination as the honeybee apparently does, but the short period of time required by the parasite to deposit a group of from six to eight eggs indicates the probability of the eggs passing the spermatheca duct so rapidly during oviposition that all of the eggs do not receive a sperm.

The writers believe also that the monembryonic development of some of the eggs of a group deposited by *Platygaster hiemalis* will account for the production of single males, or males in small numbers, in a mixed brood where females predominate, and similarly for single females or females in small numbers in a mixed brood where males predominate. In *P. hiemalis* the mixed broods more commonly yield a preponderance of females. To take a case in point, a common number of parasites reared in a mixed brood is 8, with a sex ratio of 6 females and 2 males. Studies of the number of eggs deposited by females in groups at the same time permit the writers to assume that in this instance 7 eggs were deposited in this group. Of the 7 it is also fair to assume that 4 were inseminated and 3 were not inseminated. If 2 of the 7 eggs became aborted in the course of development, and these 2 represented a fertilized and an unfertilized egg, there would remain 3 fertilized and 2 unfertilized eggs to develop successfully. The 6 females and 2 males of the mixed brood taken as an example would therefore have been produced by the twinning of each of the 3 fertilized eggs and the monembryonic develop-

[8] This fact can be readily ascertained with accuracy by examining the eggs of a group deposited by the parasite. All the eggs of such a group are identically fixed and stained, and their smallness of size permits several eggs of the group to be contained side by side within a single section cut 6µ thick. Under these conditions some of the eggs of a group show a sperm within while others show no sperm.

ment of the 2 unfertilized eggs; assuming, of course, that the fertilized eggs produced females and the unfertilized eggs produced males. If there had been 3 males instead of 2 to account for from the 2 unfertilized eggs, 1 of the eggs might have developed twins while the other developed a single male by the process of monembryony.

Admittedly, the brood taken above as an example is only one of many ratios of the sexes in which the adult parasites are bred from host puparia. But just as the number and the sex of the individuals reared from different host puparia are found to vary, so will the factors underlying the development of the individuals of a brood vary. These factors are (1) the number of eggs in the group deposited in the host, (2) the number of eggs inseminated, (3) the percentage of the eggs becoming aborted, and (4) whether the eggs that develop will follow the twinning process or will develop monembryonically. A study of many combinations of these factors, as found in various sectioned host eggs and young larvæ,[9] leads to the conclusions (1) that mixed broods originate from fertilized and unfertilized eggs deposited in the host at the same time by a fertilized parasite, and (2) that the monembryonic development of some of the eggs accounts for the rearing of one, two, three, or four males or females in a mixed brood in which the number of the individuals of the opposite sex predominates.

## BLASTULA TO LARVA STAGES

To follow the detailed development of the parasites from the blastula to the larva stages is in itself an extensive problem, and only a very general account of this development can be given here.

The parasites continue their development from the blastula stage during the fall months (Pl. 4, D, E), and early winter finds them in the advanced embryo stage in which they exhibit larval characteristics (Pl. 4, F; Pl. 5, A, B). During this interval all of the embryos which have arisen by the twinning process become separated structurally from each other, although a twin pair may still be located side by side (Pl. 5, A). Each embryo is found in an embryonic cavity the outer lining of which is the trophamnion. During the development of the embryo the trophamnion is relatively thick and contains many small (Pl. 4, E) or two large conspicuous paranuclear masses. The parasites pass the winter as well-formed embryos distributed between the fat bodies of the host, which has meanwhile become fully grown and encased in a puparium on the wheat plant.

In spring the parasites continue their development. The embryos straighten out from their previous U-shaped form and are recognized as young larvæ. While this development is taking place, the trophamnion becomes relatively thinner and its paranuclear masses are absorbed until it is represented merely by a very thin membrane surrounding the young larva (Pl. 4, G).

In the latter part of spring, the young larvæ rupture the trophamniotic membrane and begin to feed upon the body content of the host. By the time the entire content of the host is devoured, the larvæ are full-grown. The larvæ remain within the body wall of the host during early summer, when each larva forms a chamber or cell in which it transforms to a pupa. The formation of the pupal chambers, which correspond in size to that of the larvæ, distends the body wall of the host abnormally, the

[9] The preparations originated from host eggs which had been oviposited in by parasites under laboratory controlled conditions. The parasites were reared to the advanced blastula stage in the laboratory.

host integument becoming so thin that the outlines of the parasite chambers can readily be discerned (Pl. 1, D).

The parasites remain in their chambers in the pupa or adult stage throughout the remainder of summer. They emerge as adult parasites from the host carcass and puparium in early fall, to deposit their eggs in eggs of the Hessian fly, a brood of which emerges at the same time.

## SUMMARY

(1) *Platygaster hiemalis* develops both monembryonically and polyembryonically in the Hessian fly, the adult parasites emerging in late summer from the host puparium. An average of 6.31 individuals, often of both sexes, is bred from each puparium.

(2) The female parasite deposits a group of from four to eight eggs at one oviposition in the egg and occasionally in the young larva of the host during the fall of the year. Some of the eggs of the same group are inseminated while others are not inseminated.

(3) During maturation two polar bodies are formed in the egg. These unite to form a single polar nucleus in the anterior region of the egg. Maturation is identical in fertilized and unfertilized eggs.

(4) After maturation the female pronucleus fuses with the male pronucleus to form a cleavage nucleus, which becomes located in the posterior part of the egg. The female pronucleus of an unfertilized egg is similarly found in the posterior region.

(5) Part of the egg containing the cleavage nucleus then becomes differentiated from the remainder of the egg. This differentiated part is the embryonic region, which together with its contained cleavage or embryonic nucleus gives rise to one or two embryos. The remainder of the egg, containing the polar nucleus, is homologous to the trophamnion and paranucleus of previously described polyembryonic insects. Its function is to nourish the embryos until they are young larvæ and can feed for themselves upon the host.

(6) The embryonic nucleus divides to form two and then four embryonic nuclei. At the same time the polar nucleus divides to form two polar nuclei or paranuclear masses and these divide to form four such masses.

(7) The embryonic region of some of the eggs or parasite bodies then divides to form two embryonic regions, and one region together with two of the four paranuclear masses becomes separated from the other, although both may continue development side by side for some time, being held together by host tissues. This division of the parasite body results in the formation of twin germs, each of which develops directly into a blastula, then into a late embryo stage, and finally into a parasite larva.

(8) The embryonic region of other eggs does not divide. Such eggs develop a single parasite by the monembryonic process which is similar to that described for other platygastrids.

(9) Approximately one-third of the eggs deposited do not develop beyond the cleavage nucleus stage, probably because they fail to become invested by host tissue.

(10) The twinning development in insects here described for the first time is a simple type of polyembryony. On the other hand, the monembryonic development of this parasite is very highly specialized. Since

*Platygaster hiemalis* exhibits both types of development, it furnishes a clue to the origin of polyembryony in insects.

(11) It is believed that the monembryonic development of some of the eggs, and the fact that some of the eggs of a group are inseminated while others of the same group are not, will account for the origin of mixed broods of the parasites, and the occurrence of single individuals of a sex different from that of the others of the brood.

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## PLATE 1

*Platygaster hiemalis*

A.—Outline of egg of Hessian fly showing two groups of Platygaster eggs deposited within. × 460.

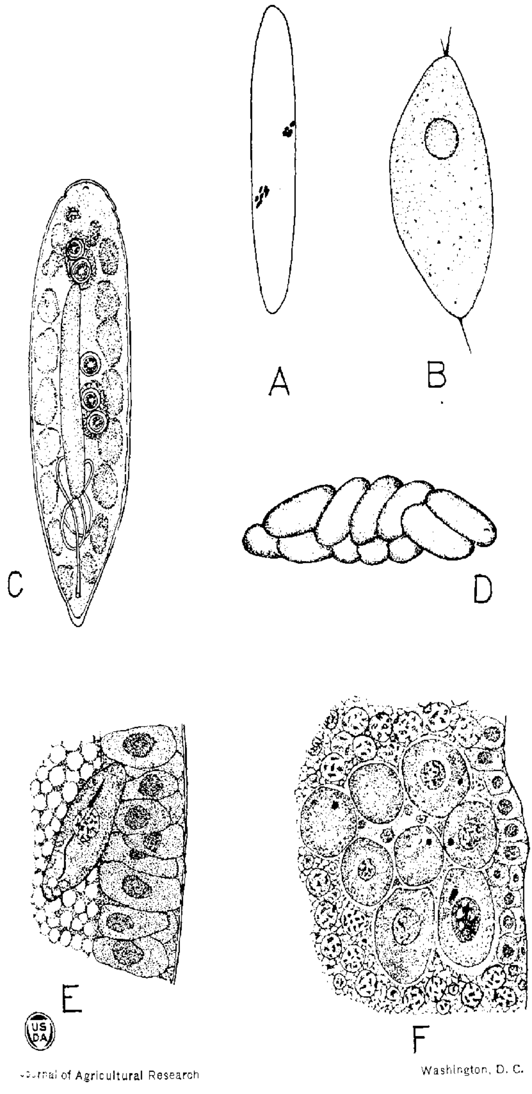
B.—Preoviposited egg of *Platygaster hiemalis*. Note the membranous process at each end and the egg nucleus. × 2,300.

C.—View within a larva of the Hessian fly, showing five Platygaster parasites in the blastula stage. × 35.

D.—Host carcass containing 11 cocoons of Platygaster. × 13.

E.—Parasite egg immediately after oviposition, with nucleus and sperm. Egg was deposited in the yolk and under the blastoderm of the host egg. Compare size of egg with that of blastoderm cells. × 2,200.

F. Portion of a section through a host egg cut across a group of eight parasite eggs which were deposited at one time. Five of the eight eggs show sections of the sperm. × 2,200.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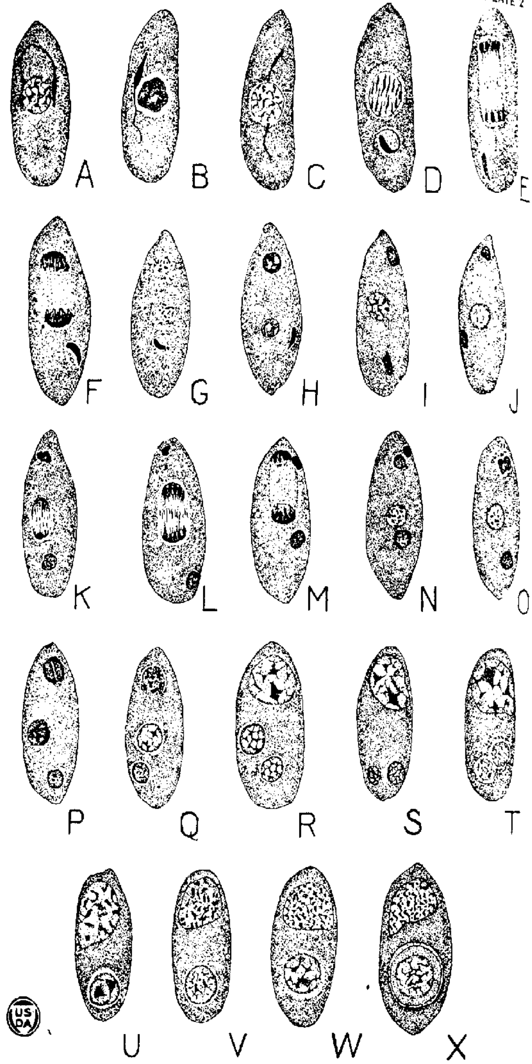
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## PLATE 2

*Platygaster hiemalis*

All figures drawn 2,255 times natural size.

A.—Egg one hour after deposition, with nucleus and sperm.

B.—Egg about one and one-half hours after deposition. Nucleus condensing.

C.—Egg 2 hours old. Chromosomes of nucleus are again evident.

D.—Egg 4 hours old. Nucleus is at prophase and sperm is curled.

E.—Egg about 4 hours old, showing first maturation spindle. The sperm or male nucleus is spindle-shaped.

F.—Like figure E but at a slightly later stage. The anterior part of the spindle becomes the first polar body and the posterior part becomes the oocyte nucleus of the second order.

G.—Egg showing first polar body which remains in the egg, oocyte nucleus, and male nucleus.

H.—Like Figure G but at slightly later stage.

I.—Egg about six hours after deposition. Polar body condenses to become an irregularly shaped mass of chromatin while the oocyte nucleus of the second order expands slightly.

J.—Like Figure I, but at slightly later stage.

K.—Egg about 7 hours old, showing the first polar body, second maturation spindle, and male nucleus.

L.—Seven-hour-old egg.

M.—Second maturation about complete. The anterior part of the spindle becomes the second polar body while the posterior part becomes the female pronucleus.

N.—Egg eight hours after deposition. The first and second polar bodies are found close to each other in the anterior end of the egg, which is known as the polar region.

O.—Egg 10 hours old. The first and second polar bodies approach each other. Female pronucleus is in center of the egg and male nucleus is usually in the posterior or embryonic region of the egg.

P.—Slightly later stage than Figure O. Polar bodies are fusing.

Q.—Egg about 11 hours after deposition, with a single polar nucleus (two fused polar bodies) in anterior end. The male and female pronuclei expand.

R.—Egg 12 hours old. Polar nucleus elaborates in anterior end of the egg.

S.—Like Figure R, but male and female pronuclei are found close to each other in the posterior region of the egg. The male pronucleus is always smaller than the female pronucleus.

T.—The male and female pronuclei are about to fuse.

U.—Fusion of pronuclei in posterior region of egg. The chromatin of the polar nucleus breaks up.

V.—Parthenogenetic egg 24 hours old showing polar nucleus, and segmentation nucleus which has maturated twice like fertilized egg.

W.—Fertilized egg about 24 hours old, with polar nucleus showing particles of chromatin scattered over a reticulum and segmentation nucleus in a resting stage. The egg is usually surrounded by host tissue at this stage.

X.—Egg from 1 to 2 days old. During this interval the embryonic region containing the segmentation nucleus becomes differentiated in the posterior part of the egg.

# PLATE 3

## *Platygaster hiemalis*

A. Like X on Plate 2, but the egg has increased in size and is henceforth known as the parasite body. During the one to two day interval the polar nucleus divides to form two paranuclear masses. The polar region and the part of the parasite body surrounding the embryonic region is known as the trophamnion. ×2,200.

B.—Section of a parasite body between two and three days after oviposition. The segmentation nucleus divides to form two embryonic nuclei. ×2,200.

C.—Parasite body about 3 days old, showing two embryonic nuclei in the embryonic region. One of the paranuclear masses begins to migrate in the trophamnion toward the embryonic region. ×2,200.

D.—Parasite body four or five days after oviposition. The two embryonic nuclei of the previous stage have divided to form four embryonic nuclei. ×2,200.

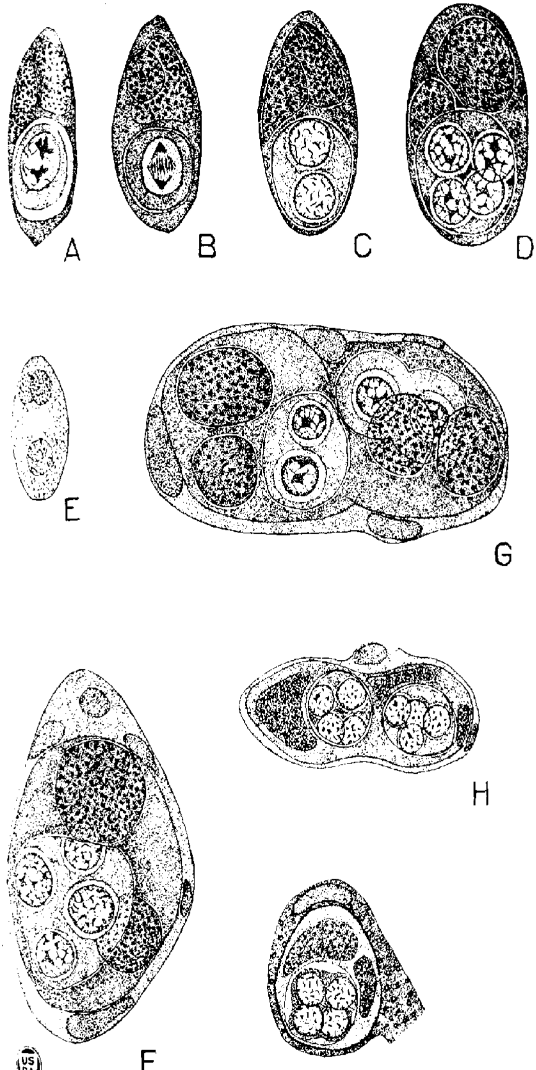
E.—Section of an aborted and degenerating egg. ×2,200.

F.—Composite drawing of two sections of a 6-day-old parasite body showing four embryonic nuclei in the embryonic region. One of the two paranuclear masses is shown lying in the trophamnion beneath the embryonic region. The parasite body is entirely surrounded by a cyst of host tissue.

G.—Section through the twinning stage of the parasite body, which takes place about six days after oviposition. The embryonic region has divided, two of the four nuclei passing to each half of the twin embryonic regions. The division of the two paranuclear masses forms four similar masses. Two paranuclear masses and one of the embryonic regions with its two nuclei thus form a germ, and the twin germs become separated structurally after the trophamnion infiltrates between the two embryonic regions. ×2,200.

H.—A twin germ surrounded by host tissue with the two embryonic regions still encased in a common trophamnion. ×1,100 (half the magnification of the preceding figures).

I.—Parasite body which is developing monembryonically while encased in a part of the host salivary gland. Four of its eight embryonic nuclei are shown in this section. ×1,100.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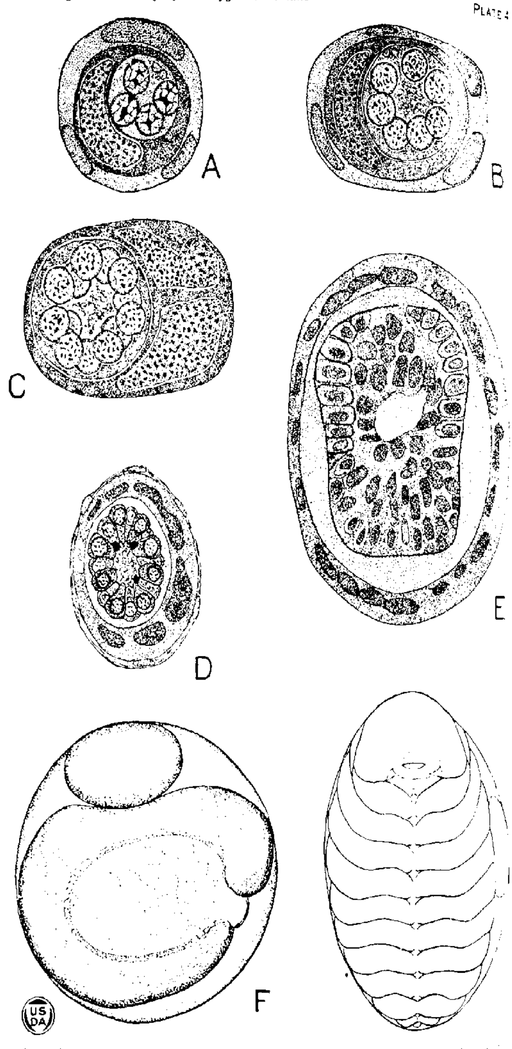
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## PLATE 4

*Platygaster hiemalis*

A.—One germ of a twin that has become disjoined from the other. It is surrounded by host tissue and its embryonic region contains four nuclei. One paranuclear mass is shown in the trophamnion. ×1,100.

B.—Embryo in the early blastula stage containing 16 embryonic nuclei, of which 7 are shown. ×1,100.

C.—Monembryonically developing egg in 32-cell blastula stage with two paranuclear masses in the trophamnion. Host tissue omitted. ×1,100.

D.—Blastula stage of embryo. Note the breaking up of the paranucleus into smaller masses. ×550.

E.—Embryo at the time of formation of germ layers. ×550.

F.—En toto drawing of U-shaped embryo within its trophamnion. One large paranuclear mass is located above it. ×108.

G.—Outline drawing of a parasite larva with paranucleus. The trophamnion has become reduced to a thin membrane. ×60.

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## PLATE 5

*Platygaster hiemalis*

A.—Photomicrograph of twin embryos which have developed side by side between fat bodies of the host. The embryos are typically U-shaped and slightly advanced in development of the one shown at F on Plate 4. × 140.

B.—Photomicrograph of a section through three embryos in one end of a host larva. Note the thickened trophamnion of the embryo on the left. × 116.


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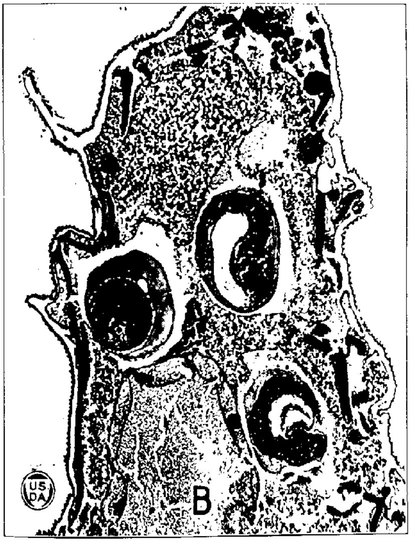
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# PATHOGENICITY OF OPHIOBOLUS CARICETI IN ITS RELATIONSHIP TO WEAKENED PLANTS[1]

By H. R. Rosen and J. A. Elliott, *Arkansas Agricultural Experiment Station*

In the United States the "Australian take-all" of wheat was first reported in 1920[2] (*6*),[3] from New York. The following year it was discovered by the writers in Arkansas.[4] As the field observations strongly suggested that the fungus, *Ophiobolus cariceti*, found on diseased wheat, was a weak parasite, attacking plants which were more or less unthrifty, experiments were undertaken to obtain more information on this point.

When Ophiobolus was first found in this country it was regarded with such alarm that gasoline was poured over the infested area and the plants burnt (*6*). This measure doubtless was justified inasmuch as the disease had been considered a serious one by investigators in Australia and in European countries, and the discoverers may have had in mind the possibility that the disease had been recently introduced into this country and was confined to a small area.

In May, 1921, Mr. O. Pool, a farmer living about 4 miles west of Fayetteville, Ark., brought into the laboratory diseased wheat showing marked blackening at the bases of the culms. (See Pl. 1 and 2.) The plants were much stunted, and the roots were largely dead and bunchy, with abnormal development of woolly root hairs on parts adjoining the stools. Blackish crusts of mycelium surrounded bases of culms, and extended into the enveloping leaf sheaths, and, finally, black, beaked, fruiting bodies, quite noticeable under a hand lens, were found submerged in the lower parts of the sheaths; when these were examined under the microscope, asci and spores, typical of Ophiobolus, were found.

## FIELD OBSERVATIONS

The writers immediately went to the field from which the plants had been obtained and saw 11 acres of very sickly looking wheat. The field was undulating, with elevated spots on the eastern and western borders and a low area in between, which, according to Mr. Pool, became a good sized pond during rainy seasons, being flooded with water for two or three months at a time. The soil even on the elevations appeared poorly drained, with crawfish holes very noticeable. It is classed as Gasconade silt loam, a type, grayish white in appearance, which is usually poorly drained in this region, often giving an acid reaction, as this soil did.

The stand of wheat as a whole was poor (Pl. 3), the heads, even on the best plants, were undersized (by the end of May, wheat is usually well developed in this latitude), and bare spots were noticeable throughout the field. Many of the plants were stunted, 6 and 12 inch plants were bearing heads, and were either dead or about to die;

[1] Accepted for publication June 25, 1923.

[2] The disease of wheat first found in Madison County, Ill., in 1919 and at one time considered as "Australian take-all," or "so-called take-all," is now considered by McKinney, Eckerson, and Webb (*8*) as a "rosette disease" associated with intracellular bodies comparable to those found in various mosaic diseases. Stevens (*11*), in considering the footrot stage of the disease, has concluded that this is due to Helminthosporium.

[3] Reference is made by number (italic) to "Literature cited," p. 358.

[4] U. S. Department of Agriculture. Bureau of Plant Industry. Plant Disease Survey. Wheat. The take-all survey. *In* U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul., v. 5, p. 4. 1921. (Mimeographed.)

the leaves were yellowish, straw colored, markedly withered, and harbored any number of different fungi. Cladosporium and Alternaria spp. were so common on the leaves and heads as to give the plants a distinct, mouldy appearance. A careful survey of the field made on this trip and on subsequent visits showed Ophiobolus present at the base of many plants growing on the elevations. Numerous plants growing in the lower parts of the field appeared just as sickly as those on the knolls, but in contrast showed no evidence of the presence of Ophiobolus. There was but slight dark discoloration at the crown, and no black fungus crusts or fruiting bodies were found. The variety of wheat grown was Marvelous. It is a soft, winter wheat, not infrequently used in this section. The seed had been procured from a local grower who had obtained good yields from the same strain in previous years. This field had not grown wheat previously for 20 years and had been used as a pasture of red top and timothy (largely red top) for the past 12 or 14 years.

Mr. Pool had also another field of wheat, about 500 yards south of this, which had been grown from the same lot of seed, but this field, in contrast to the one just described, showed normal, healthy looking plants. it gave one of the best yields in the county. The soil here was of a different type, darker in color, well drained, and it had received an application of manure the previous year. It seems proper to conclude that as far as these two fields are concerned the seed played no part in the introduction and development of the disease. Furthermore, it is evident that as far as ecological factors are concerned the only known difference was that of soil condition.

Shortly after Ophiobolus was found on this farm it was discovered by the senior writer on another, near Prairie Grove, about 10 miles away. Here also the field was undulating and about 11 acres in size, but, unlike the first, Ophiobolus was confined entirely to certain spots at the north end. This portion represented the highest part of the field, the soil of which in color, texture, moisture content, and acidity was comparable to the Pool field. Ophiobolus was sharply confined to spots in a 3-acre area which had previously been used as a peach orchard. A fence row had formerly delimited the southern edge of this area and had been removed shortly before the land had been prepared for wheat the previous fall. The whole 11-acre field had then received the same treatment, and the drill rows had been run north and south, extending across the area that had previously been in peach trees and continuing down through the remainder of the field. The remaining 8 acres had been used for wheat in 1920, corn in 1919, and clover for 3 years previous to that. As compared with the north end of the field, the stand of wheat on this portion was thicker, the plants were larger, and the heads better filled. The soil was considerably better physically; it appeared well drained, there were no crawfish holes, and the subsoil instead of being a stiff clay[6] of a grayish color was more or less friable, reddish brown, and not water-soaked.

As just stated, Ophiobolus was found only in the 3-acre portion at the north end. Here the wheat as a whole was much poorer, and the general appearance of the plants closely resembled that on Mr. Pool's farm. Here also many stunted and badly diseased plants showed no signs of Ophiobolus. One of the worst infested spots in the field was a strip of

[6] The writers wish to express their thanks to Mr. R. H. Austin of the agronomy department, University of Arkansas, for taking samples of the soil and subsoil.

land running along its entire width through which the fence row had previously passed; part of the fence still existed to the west of the wheat field. This row marked the southern edge of an incline, and to the south of it the area was quite level throughout, and of a different type of soil, as above stated.

While Ophiobolus, both perithecia and mycelium, could be found scattered in the north end, a very careful search beyond the edge of the incline to the south failed to reveal a single plant with symptoms of Ophiobolus. The field was revisited several times; on one occasion the writers were accompanied by two other plant pathologists, Dr. A. G. Johnson and Mr. H. H. McKinney, and no evidence of attack by Ophiobolus could be found by anyone south of the region where the fence row had existed. As will be fully described later, a number of wild grasses were also found badly attacked by Ophiobolus in this same strip. In spite of the excellent opportunity for infection taking place on both sides of this strip, the drill rows extending through from the poor soil to the good soil, infections were sharply confined to the wheat growing to the north upon the poor soil. Here, as on the Pool farm, there appeared to be a close correlation between the presence of weak, sickly plants and the pathogenicity of Ophiobolus.

## OPHIOBOLUS CARICETI ON WILD GRASSES

In addition to wheat, the following grasses have been found attacked: *Festuca octoflora*, *Festuca elatior*, *Bromus secalinus*, *Hordeum pusillum*, and *Chaetochloa geniculata*. Dr. A. G. Johnson first detected signs of Ophiobolus on a wild grass in the diseased wheat fields near Prairie Grove, previously mentioned, and while no fruiting bodies were observed at the time, a careful inspection by the senior writer of material subsequently gathered on the same field revealed typical fruiting bodies on two wild hosts, *Festuca octoflora* and *Bromus secalinus*. (Pl. 4.)

In the same strip of soil where the fence had previously existed, as well as in other spots in the north end of the field, many plants of *Festuca octoflora*, *Bromus secalinus*, and *Hordeum pusillum* were found showing symptoms similar to the diseased wheat in the same vicinity. A thorough search for Ophiobolus on wild grasses near take-all diseased wheat on the Pool farm also brought to light many diseased plants of *Festuca octoflora* (a common grass in this locality), on some of which perithecia of Ophiobolus were found.

As the presence of Ophiobolus on these wild grasses strongly suggested an organism more or less endemic in nature, a search was made for it in regions where wheat had not been grown. It was shortly after detected on unthrifty plants of perennial foxtail (*Chaetochloa geniculata*), growing in a few water-logged areas on the campus of the University of Arkansas. No further search was made, but as no wheat had ever been grown on the campus (used as such for about 50 years) there is no reason for doubting the endemic nature of Ophiobolus on this native grass.

## IDENTITY OF THE ASSOCIATED ORGANISM

The question as to whether or not the species of Ophiobolus which attacks wheat is the same as that on the wild grasses was determined by a careful comparison of perithecia, asci, spores, and mycelium found on the different hosts. An illustration of the close morphological agreement may

be seen by the following measurements of asci and ascospores found upon three different hosts.

| | Asci (μ). | Ascospores (μ). |
|---|---|---|
| Wheat | 75.0 to 105.0×10.5 to 13.5 | 60.0 to 90.0×3.0 to [illegible] |
| Chaetochloa | 80.0 to 100.0×10.2 to 13.0 | 61.0 to 87.0×3.0 to [illegible] |
| Festuca | 85.0 to 100.0×10.0 to 13.0 | 61.0 to 88.0×3.0 to [illegible] |

These measurements are also in close agreement with those given by Fitzpatrick, Thomas, and Kirby (*4*). As these authors have not considered the species of Ophiobolus previously described which is found on wild grasses in this country, it seems desirable to present briefly the data obtained by the senior author on this phase of the subject.[6] Of the species of Ophiobolus that have been described as occurring on wild grasses the following deserve consideration: *O. Andropogonis* E. & E., and *O. Festucae* Tracy and Earle. Through the kindness of Dr. F. J. Seaver of the New York Botanical Garden type material of *O. Andropogonis* was carefully examined and compared with Arkansas material. As far as measurements of asci and ascospores are concerned, Ellis and Everhart's (*3*) species might readily be taken for underdeveloped material of *O. cariceti*, but the perithecia with large beakless osteoles, the absence of any blackish mycelium on the host tissue in which the perithecia are buried, and the apparent saprophytic nature of the fungus, are sufficient to mark it as distinct. Examination of co-type material of *O. Festucae* Tracy and Earle (*13*) found in the Missouri Botanical Garden shows two different fungi present, but the one, doubtless, which was described possesses asci and ascospores very much like those to be found in Ophiobolus species. However, the perithecia instead of being beaked or papillate, open by means of a cleft in the host epidermis. The fungus belongs to the genus Lophodermium and perhaps is close to *L. arundinaceum* (Schrod.) Chev. var. *alpinum* Rehm. Other species of Ophiobolus which have been considered, but which, judging from the descriptions, are very different from *O. cariceti* are: *O. eucryptus* (B. & Br.) Sacc., *O. leptospermus* (Speg.) Sacc., *O. trichisporus* E. & E., *O. medusae* E. & E., *O. paludosus* (Fritz.) Sacc. & D. Sacc., *O. herpotrichus* (Fr.) Sacc., *O. coffeatus* (*Berk.*) Sacc., *O. stictisporus* (Cook & Ellis) Sacc., *O. littoralis* (Crouan) Sacc., *O. culmorum* (Crouan) Sacc., and *O. helicosporus* (B. & Br.) Sacc.

## PATHOGENICITY OF OPHIOBOLUS

As all the field observations suggested that Ophiobolus attacked weak, poorly growing plants, it seemed worth while to test the effect of different fertilizers in a field where Ophiobolus was present. For this purpose 4 acres of the field in which Ophiobolus existed were set aside by Mr. Pool for our use. The 4 acres chosen consisted of two areas of similar size, one in the southeast corner and the other in the northwest corner of the field. These parts were selected because wheat attacked by Ophiobolus had been found to be most abundant on them and the fungus appeared equally distributed throughout. The chosen areas were divided into 1-acre plots, each of which received the following: Plot 1, no treatment; plot 2, 10 tons of manure; plot 3, 1 ton of burnt lime; plot 4, 400 pounds of commercial fertilizer of a 4-8-3 formula. The manure, lime, and commercial fertilizer, with the exception of sodium nitrate, which was applied the following March, were disked in at the same time, in the fall, and all the plots otherwise received the same treatment throughout.

[6] We are indebted to Dr. E. A. Burt for the use of the splendid library and mycological herbarium of the Missouri Botanical Garden.

In order to ascertain any difference in varietal susceptibility in wheat, as well as the pathogenicity of Ophiobolus on *Festuca elatior*, each plot was divided into four equal parts, sown to grass and wheat. One of these parts in each acre plot was sown to Festuca, another to Pool's Marvelous (wheat which had been grown on this field the year previous), another to Alabama Blue Stem, and the remaining equal part of each plot to Marvelous.

The plots were visited [7] frequently and notes kept of their appearance at different times. The first marked differences were noted on December 10, 1921. Among the notes taken on that day are the following: Manure plot, plants 4 to 5 inches tall and of good color; stand is good, Alabama Blue Stem showing up the best. Lime plot, much like the untreated plot; stand is poor, plants are off color and lacking in vigor. Commercial fertilizer plot, stand is excellent; the plants are of good color and vigorous. Untreated plot, stand poor; plants are undersized, spindling, and off color.

From December on through the winter and spring the same differences prevailed. On April 18, when the plots were photographed (see Pl. 5, A and B), those treated with manure and commercial fertilizer showed excellent growth as compared with the untreated and limed plots. A few of the plants in the manure as well as in the commercial fertilizer plots appeared undersized, but it was impossible to tell whether this was due to improper distribution of the manure and fertilizer in the vicinity of such plants, or to some other cause. On June 8, when the heads were almost mature, it was possible to make actual counts of the number of plants which clearly possessed symptoms of Ophiobolus. The amount of infection was as follows: Untreated, 80 per cent; manure, 45 per cent; commercial fertilizer, 7 per cent; lime, 95 per cent. When the wheat was harvested and threshed the following yields were obtained:

| Variety. | Yield from untreated plot. | Yield from plot treated with manure. | Yield from plot treated with commercial fertilizer. |
|---|---|---|---|
| | *Bushels.* | *Bushels.* | *Bushels.* |
| Pool's Marvelous | 0.8 | 3.0 | 5.5 |
| Alabama Blue Stem | 1.2 | 5.3 | 5.0 |
| Marvelous | 1.1 | 2.0 | 3.3 |

The lime plot was a total failure; the plants were so stunted and the stand so poor that it was considered useless to attempt to harvest the crop. The figures shown above represent actual yields of each one-fourth-acre plot. The one-fourth acre of Festuca which was included in each plot is not figured, since the stand on all was poor, weeds having taken possession of the soil before the plants had made any growth. Figuring on the basis of yield per acre and averaging the three varieties, the untreated plot yielded 4 bushels, manure plot 14.3 bushels, and commercial fertilizer 18.4 bushels (Pl. 5). The yield from the manure plot is above the average for the State, while that from the commercial fertilizer was considered so phenomenal by the farmers in the vicinity that it attracted

[7] The writers are greatly indebted to Mr. R. F. Crawford, who spent much of his time in seeing that these experiments were properly carried out, and in keeping careful notes on the various plots.

a great deal of attention. The yield of the control plot was comparable to that obtained by Mr. Pool on the same area the previous year.

## DISCUSSION

The writers have no satisfactory explanation to offer for the presence of the Ophiobolus on some of the plants of the manure as well as the commercial fertilizer plots, but whatever such an explanation may be, it is clear that irrespective of the presence of the fungus the yields on these plots as compared with those on the untreated and lime plots were so satisfactory that for practical purposes these treatments, particularly the commercial fertilizer, may be considered as having almost completely controlled the disease. The percentage of infection on the manure and commercial fertilizer plots as compared with that on the check and lime plots was so strikingly different that there can be no question that the parasitism of Ophiobolus was greatly inhibited. Why commercial fertilizer gave better control than manure can not be answered at this time, but it is perhaps safe to conclude that control may be not entirely a matter of a sufficient amount of nutrients.

The pathogenicity of *Ophiobolus cariceti* (*O. graminis*) on healthy plants has been questioned by various investigators previous to this time. From Stevens' (*10*) excellent summary of the literature on wheat footrots, including Australian take-all, the following references may be noted: Pearson (*9*) in 1888 decided that this is a poverty disease, "and that the fungus which causes take-all attacks mainly such crops as are insufficiently nourished." Tepper (*12*) in 1892 said that "Take-all is nothing else than starvation of the crop." McAlpine (*7*) in 1902 stated that "Take-all largely depends on the nature of the season and the mechanical condition of the soil. * * * If the soil is neither too dry * * * nor so wet as to cake * * * and contains sufficient plant food * * * then the take-all will not appear." Voges (*14-18*) in a number of articles running over a period of years up to 1914 concludes that Ophiobolus is not the primary cause of the disease, but, judging from his descriptions, it may be doubted whether he had studied the Ophiobolus disease which is described by McAlpine and others as common in Australia. Various other investigators who have associated Ophiobolus with this disease have concluded that conditions which tended to weaken the plants, such as frost, wet weather, poor soil, etc., rendered the plants susceptible to attack by this fungus. Most of them, however, rely almost entirely on field observations, and little or no attempt has been made to study these relationships under rigid experimental conditions with adequate controls.

Contrary to the opinion that Ophiobolus attacks only weakened plants, the recent paper by Kirby (*5*) presents the view that the fungus is a vigorous parasite capable of attacking healthy plants, and cites the work of other investigators who express or imply similar views. Inasmuch as Kirby's work is much more thorough and exact than that of any of the others it may suffice to analyze his work. Using pure cultures of *Ophiobolus cariceti* growing on wheat kernels, he inoculated 156 pots of wheat at planting time and noted that at maturity the plants in all the inoculated pots showed typical symptoms of take-all, while no plant in any of the 72 control pots exhibited such symptoms. From the inoculated plants he then recovered the same fungus. As far as this evidence is concerned, there appears to be no doubt that *Ophiobolus cariceti* can attack wheat and other grasses, but the question that one asks after reading his paper carefully is, What was the condition of the wheat, inoculated or uninocu-

ited? Anyone who has tried to grow wheat in 5-inch pots, as Kirby id, under ordinary greenhouse conditions for considerable periods of me, knows how difficult it is to avoid weak, spindling, under-developed ants, and when one turns to his photograph (Pl. II, fig. c) labeled Healthy and diseased plants after four months' growth," it is noted that he pot of "healthy" plants is so full of drooping, withered, and dead eaves as to make the plants appear decidedly unhealthy. Kirby be-ieves that the fungus also produces a seedling blight[8] for, speaking of field bservations, he says (*p. 68*), "An accurate determination of the amount f damage caused by take-all was impossible because many of the plants vere killed in the seedling stage." And farther on, under "Symptoms," e says, "The writer has not had the opportunity to study the disease hrough all its early stages in the field." But what about the early tages in the inoculated pots? Although he inoculated when the seed vas sown he notes no Ophiobolus symptoms until ten and a half weeks after inoculation (*p. 74*). His work, therefore, can not be said to pre-ent a clear picture of the relationship of attack by *Ophiobolus cariceti* to he condition of the host.

The increase in the percentage of infection on the lime plot in the writers' experiment is in full accord with the work of Kirby and of others, although, as we have already noted, Ophiobolus was able to attack plants rowing in acid soil. Of course the relationship of the growth of the wheat to change in acidity of the soil must be considered, for it is now well established (*2*) that growing plants often cause a marked change in he hydrogen-ion concentration of the media in which they are growing.

## CONCLUSION

As the results obtained by the writers in controlling this disease involve only one year of experimental work, it would be improper to make any definite recommendations at this time, but inasmuch as these experiments fully confirm numerous other observations made by the writers and by others, there seems to be sufficient ground for concluding that *Ophiobolus cariceti* confines its attack to weakened plants. The discovery of this organism in such widely separated regions as New York, Oregon, Indiana, and Arkansas (*5*) suggests that it is present over a large part of the country, and that it has been overlooked because it is of little economic importance.

## SUMMARY

*Ophiobolus cariceti* was discovered in two wheat fields in Arkansas and on the campus of the state university. Wheat, *Bromus secalinus*, *Chaetochloa geniculata*, *Festuca octoflora*, *Festuca elatior*, and *Hordeum pusillum* were found infected.

Infection appears to be confined to weakened plants. Lack of proper nutrients and water-logged soils in particular were found to be conducive to attacks by this fungus.

[8] Under unfavorable conditions it is entirely conceivable that seedlings also become susceptible, and McKinney's report[9] of a seedling blight in soil kept at temperatures near 22° and 24° C. would indicate that soil temperatures are important in the development of this disease. From Dickson's work (*1*) it is clear that wheat requires a rather low temperature for good growth. He writes (*p. 840*): "While spring wheat germinated more rapidly at soil temperatures of 24° to 28° C., the germination was more uniform and stronger plants resulted at the lower temperatures—about 8° to 16°. The greatest development of roots as well as tops occurred at the lower soil temperatures. The earliest maturing, most stocky, and best filled plants resulted at soil temperatures of about 16°." As far as winter wheat is concerned, he says: "The cardinal temperatures for the development of Turkey winter wheat were, in general, about 4° C. below those for spring wheat."

[9] McKinney, Harold H. TAKE-ALL AND FOOT-ROT INVESTIGATIONS. *In* U. S. Dept. Agr. Bur. Plant Indus. Cereal Courier, v. 14, p. 24-25. 1922 (mimeographed).

In an experiment involving the use of lime, manure, and commercial fertilizer on different plots of a field in which Ophiobolus had previously been discovered, found attacking a large area of growing wheat, it was found that commercial fertilizer almost completely eliminated the disease, manure decreased the percentage to a considerable degree, and lime increased the incidence of infection.

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PLATE 1

Wheat plant grown on the Pool farm, attacked by Ophiobolus. Note discolored and dead roots and bases of culms and "fuzzy" appearance of roots due to abnormal growth of root hairs. Photographed by senior writer, May 17, 1921.


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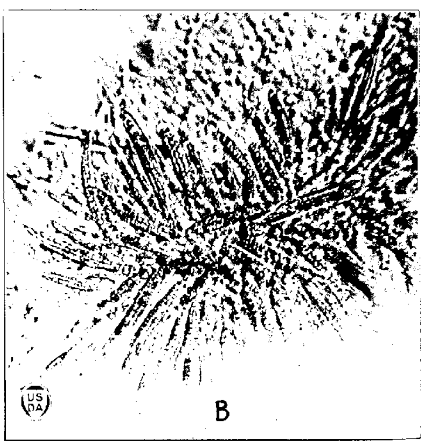
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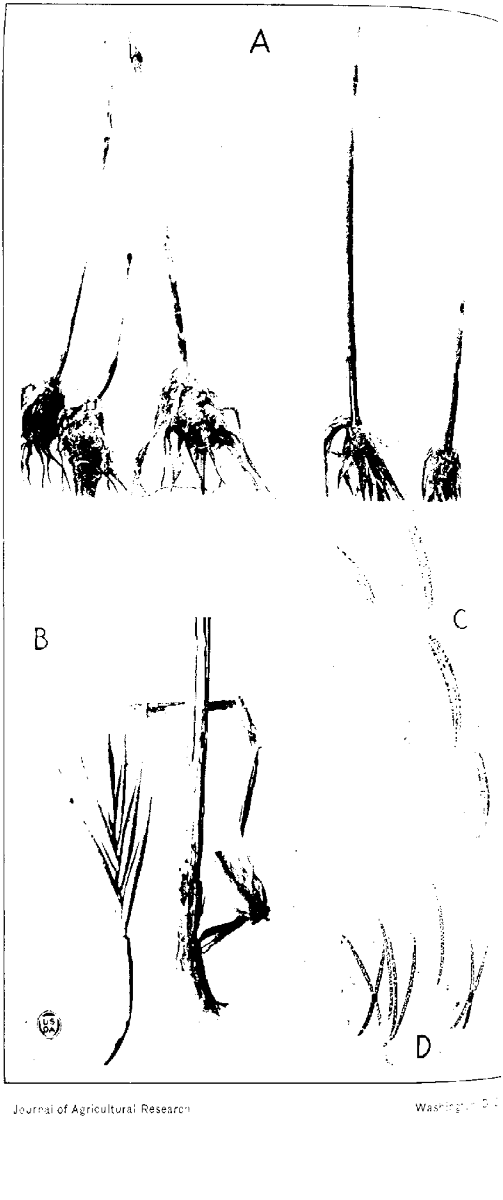
PLATE 2

A.—Plant attacked by Ophiobolus on the left, healthy plant on the right, all obtained on the Pool farm and photographed at the same time as Plate 1.

B.—Photomicrograph of a cluster of asci of *Ophiobolus cariceti* from *Chaetochloa geniculata*. The diseased host was discovered on the campus of the University of Arkansas. Magnified about 250 times.

PLATE 3

Two views of the Pool field in which Ophiobolus was found over a considerable part of an 11-acre area. Photographed by the senior writer, May, 1921. (Compare with Plate 5.)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## PLATE 4

A.—Three plants of *Festuca octoflora* to the left and two of *Hordeum pusillum* to the right, all badly attacked by *Ophiobolus cariceti*. (Slightly enlarged.)

B.—Spikelet of *Festuca octoflora* to the left and lower part of culm to the right, showing perithecial beaks of *Ophiobolus cariceti*. (Slightly enlarged.)

C and D.—Asci and ascospores of *Ophiobolus cariceti* from perithecia found on *Chaetochloa geniculata*. Magnified about 300 times.

PLATE 5

A.—Manure plot to the right, control plot to the left. Photographed April 18, 1922.

B.—Commercial fertilizer plot to the right, lime plot to the left. Photographed April 18, 1922.

C.—Manure plot in early June, 1922. All three photographs by the junior author.

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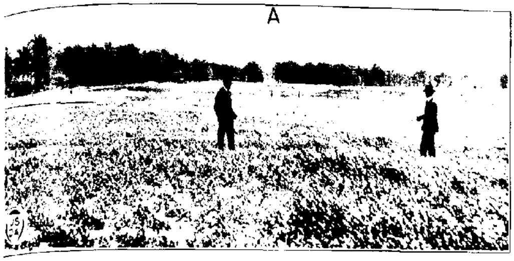

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# THE PHARYNX AND ALIMENTARY CANAL OF THE HOOKWORM LARVA—NECATOR AMERICANUS[1]

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The following facts, based on observations recently made by the writer (Pl. 1), may be given as reasons for regarding the pharynx of the full-grown larva of *Necator americanus* as somewhat in the nature of a protrusile onchium:

(1) The apex of the pharynx (*on*) in different specimens, fixed and living, varies in position from considerably behind the amphids to a slight protrusion beyond the lips.

(2) The form of the pharynx reminds one of the protrusile onchium of *Mermis*.

(3) The pharyngeal wall is of considerable thickness, such as would impart to it the rigidity necessary for puncturing, while the lumen is very narrow, as in many Tylonchs, "spear-bearing" nemas characterized by the possession of an onchium presumably evolved through conversion of a thin-walled cylindroid or prismoid pharynx into a relatively thick walled, tubular, protrusile spear.

(4) Round the front portion of the pharynx is a refractive ringlike element (*dir on*) similar to that found in nemas armed with a protrusile spear. This encircling element in such cases serves as a guide for the spear when in action. The location, form, and size of this element in *Necator* larvae harmonizes well with the supposition that the associated "onchium" is protrusile; its position accords with the limits of longitudinal motion apparently justly attributable to the "onchium" on the basis of observations made on a considerable number of specimens.

(5) The front part of the pharynx is surrounded (?) by tissue readily explainable as contractile, (*msc*?) similar to that seen in nemas having a protrusile onchium. Such muscles are usually attached to the posterior part of the onchium and to the labial cutin.

(6) The oesophagus of the Necator larva contains "salivary" glands emptying precisely as in a large number of well known nemas possessing a protrusile onchium, namely, three unicellular oesophageal glands having their nuclei located in the posterior oesophageal swelling and emptying forward through three separate ducts, two emptying into the lumen of the oesophagus near its middle, and the third extending farther forward in the dorsal sector of the oesophagus and emptying at the base of the onchium (*gl sal dsl*).

(7) Considered in the light of the known entrance of hookworm larvae into the human host through the skin, these pharyngeal and oesophageal elements fall into a harmonious group in accord with the proposal that the pharynx when acting as a mechanical puncturing organ is assisted by the oesophageal fluid, acting as a solvent, in forcing a passage through the skin.

[1] Accepted for publication May 26, 1923.

A prominent symptom connected with the penetration of hookworm larvae into the human skin is an itching sensation lasting several days. It seems quite as likely that this irritation is caused by a secretion of the larva as by its motions. Reflecting that the "bite" of many insects often, at the time, is accompanied by very little sensation of any kind, while the wound after the withdrawal of the insect's mouth parts becomes inflamed, it is an obvious inference that some substance injected into the wound causes the inflammation rather than the puncture itself. This line of observation and reasoning is in harmony with the supposition that *Necator americanus* works its way through the human cuticle partly by the aid of a solvent, which, incidentally, may account for the accompanying symptoms.

The fact that this onchium of *Necator* larvae has never been seen in action, that is, has never been seen to move in living larvae under the microscope, throws little, if any, doubt on its being protrusile, for it is well established that the examination even of thousands of specimens of nemas of various kinds possessing a protrusile spear may not enable one to make such a direct observation. It is only on rare occasions that the protrusile onchium of a nema has been seen in action, the commonest occasion being its use by the larva in escaping from the egg. Here the larva, even under the blaze and other inhibiting conditions of the microscope, sometimes remains in condition to proceed with its operations. However, many thousands of observations made by numerous competent observers, using hundreds of different species of nemas possessing a protrusile onchium, has resulted in accumulating circumstantial evidence so strong as practically to prove, in this way alone, even unsupported by other evidence, that the onchium is protrusile. This circumstantial evidence, as in the case of *Necator*, relates very largely to differences in the observed position of the onchium, but also relates to its form. On such evidence alone belief that the onchium is protrusile is practically unavoidable, since no other explanation of its form or function can reasonably be offered.

These various considerations appear to me to make it at the very least a reasonable working hypothesis that the pharynx of *Necator americanus*, at the time it reaches the condition of the "full grown larva," is modified into what may fairly be regarded as a protrusile organ.

Interesting morphological speculations with regard to nema anatomy are associated with such an hypothesis. The young larva of *Necator americanus* is said to be rhabitdiform. Now there are certain nema genera in which the rhabditoid form of pharynx, when the species are assembled and suitably arranged, may be "traced" step by step to a pharynx similar to that of *Tylenchus*, a well known genus containing both free-living and parasitic nemas and possessing a protrusile onchium used for piercing, and this fact has led to the promulgation by Marcinowski of a theory that the tylenchoid pharynx may have been evolved from the rhabditoid pharynx.

## THE AMPHIDS OF NECATOR

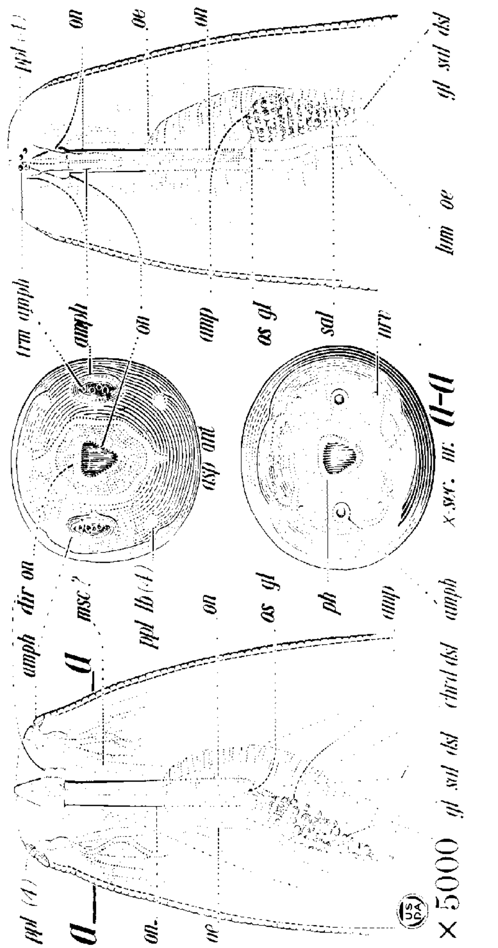
The writer's observations on the amphids on *Necator americanus* (*amph*) are not subject to the qualifications necessary in the case of the pharynx. That these organs on the front of the head of *Necator* fall into the group of organs known as amphids seems to me beyond question. Their number, form, exact symmetry, position, and structure are those typical of well known amphids. The nature of the amphidial

terminals (presumably nerve endings), arranged symmetrically on opposite sides of the head within the amphids (*trm*), lead to the belief that the backward processes connected with them pass to the nerve ring, although they have not been traced thither. It would be an interesting piece of work to trace them backward by means of serial sections of the adult nema.

The cells of the intestine throughout its length, and some of the cells of the body wall, are well stocked, in the young larva, with fat globules. These darken in osmic acid, those in the body wall apparently more vigorously than those in the cells of the intestine, a difference that may be connected with the relative position of the two groups of globules.

## PLATE 1

At right and left, dorsal and lateral views of the head of *Necator americanus* free living larvae when full grown; between them the front view of the head and an optical section near the line *AA*; *ppl*, one of the four labial submedian papillae; *on*, onchium or pharynx; *oe*, oseophagus; *gl sal dsl*, dorsal oesophageal gland; *chrd dsl*, dorsal chord; *amph*, amphid; *amp*, ampulla of the dorsal oesophageal gland; *ph*, pharynx; *os gl*, dorsal oesophageal gland emptying into the lumen of the oesophagus or pharynx; *msc?*, contractile fibers(?); *dir on*, guiding ring encircling the anterior portion of the pharynx; [illegible] *amph*, terminals in the amphid, presumably the end elements of nerve fibers; [illegible] dorsal oesophageal gland; *nrv*, and opposite *AA*, nerve passing to one of the submedian labial papillae; *lum oe*, lumen of the oesophagus. The dorsal and lateral sketches were made from living specimens, the front view and section from specimens fixed in Flemming's solution and mounted in glycerin jelly.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